Subgroup: Molecular Biophysics

1-Subg
Computational Modeling of Glycolipid-Glycoprotein Complexes: Methods and Applications
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The human innate immune response to a Gram-negative bacterial infection involves detection of lipopolysaccharides (LPS), also known as endotoxins, which comprise the bacterial outer cell wall. Distinct from mammalian glycolipid structures, LPS have a conserved chemical pattern that is recognized by the pattern recognition receptor complex formed by myeloid differentiation protein 2 (MD-2) and toll-like receptor 4 (TLR4). While there is a clear correlation between endotoxin acylation and elicited agonist or antagonist responses, the 3D structural basis of this relationship remains unclear and difficult to characterize experimentally. In order to explore, at atomic-resolution, the effects of a range of chemically distinct endotoxins on the structure and dynamics of their MD-2·endotoxin complexes, a series of variably acylated lipid A molecules from E. coli and N. meningitidis in complex with human MD-2 were examined. This included the development and validation of appropriate molecular dynamics force fields to study complex (lipid, carbohydrate and protein) systems. Through these computational developments, in concert with experimental data, specific structural and dynamic features that control dimerization of TLR4 molecules were identified. As dimerization is central to the release of downstream chemical mediators, the results provide a structural foundation for the ability of endotoxins to act as either agonists or antagonists of the TLR4 pathway. Additionally, methods for biologically-relevant characterization of dynamic glycolipid-glycoprotein systems will be discussed.

2-Subg
Protein-Ligand Binding by Free Energy Simulations: Issues, Successes and Failures
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The use of rigorous free energy calculations, well grounded in statistical mechanics, to predict protein-ligand binding affinities, is becoming increasingly common. The major challenge in their accurate use is often no longer the free energy methodology itself, but rather the problems of accurately representing the inter and intra-molecular energies, and ensuring sufficient sampling of the relevant configuration space. In this presentation, results related to tackling these problems will be presented for the specific case of the enzyme Dihydroorotate Dehydrogenase. This is a challenging target since small changes in ligand structure are observed to result in multiple binding modes, according to X-ray crystallography, and marked variations in hydration pattern in the protein-ligand complex. The implications of these results on the naive application of free energy calculations to protein-ligand systems with less experimental data will be discussed.

3-Subg
A Multiplex Suspension Array for Screening of Carbohydrate Binding Proteins and Influenza
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Carbohydrate-protein interactions are involved in many crucial biological processes, from protein stability, to immune response, to pathogen infection. A fast and reliable method to determine carbohydrate binding affinity and specificity was developed as an alternative to ELISA and glycan microarray screening.
Optically distinct neutravidin-coated microspheres were functionalized with biotinylated carbohydrates, to create a glycan array in suspension format. A range of fluorescently labeled carbohydrate binding agents including lectins, toxins, and viruses were incubated with the glycan suspension array. In addition to characterizing carbohydrate binding preference, simultaneous strain typing of influenza virus was performed using additional multiplex microspheres conjugated to antibodies specific for influenza A subtypes. Inactivated virus was incubated with the microspheres, detected using fluorescence-labeled anti-influenza virus antibodies, and measured by flow cytometry.
Biotinylated carbohydrates were successfully captured by neutravidin conjugated microspheres as observed by specific binding of fluorescently labeled lectins SNA-I and MAA and Cholera toxin B (CTB) subunit. Antibodies were also conjugated to microspheres allowing for multiplex analysis of influenza virus sub-type and receptor specificity of human influenza A subtype H1N1 and avian influenza A subtype H5N1. Each subtype of influenza virus bound only to those microspheres displaying carbohydrates with its specific receptor and anti-influenza A antibody. These results have been confirmed previously by other methods, including hemagglutination and glycan microarray.
This approach provides improved control over carbohydrate surface density, increased throughput for the analysis of many samples and conditions, reduced sample volumes, and better quantification.

4-Subg
Determination of Protein-Ligand Affinities by Direct ESI-MS Measurements
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The direct electrospray ionization mass spectrometry (ESI-MS) assay has emerged as a powerful tool for quantifying protein-ligand interactions in solution. The assay is based on the direct detection and quantification of free and ligand-bound protein ions by ESI-MS for solutions of known initial concentrations of protein and ligand. A brief overview of the ESI-MS assay will be presented, along with recent methodological advances that overcome the major sources of error in the binding measurements. Several examples illustrating the application of the assay for quantifying Ka values for protein interactions with carbohydrate and fatty acid ligands will be given. A high-throughput ESI-MS approach to library screening will also be presented. The ''catch and release'' ESI-MS assay involves incubating a protein with a library of compounds in solution, detecting the protein-ligand complexes by ESI-MS, activating the complexes to release the ligand, followed by fragmentation of the ligand. The identification of the ligand is based on the measured molecular weight and the fragmentation spectrum of the ligand. Collision cross section measurements also aid in ligand identification. The ''catch and release'' ESI-MS assay allows for the sensitive, rapid (<1 min analysis time) and direct detection of specific protein interactions within libraries containing upwards of several hundred compounds. An overview of the assay will be presented followed by examples highlighting the application of the assay for the discovery of carbohydrate ligands of bacterial proteins.

5-Subg
Structure Activity Relationships of Nuclear Receptor, GPCR and Kinase Modulators Revealed with Differential HDX
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Hydrogen/deuterium exchange coupled with mass spectrometry (HDX-MS) has emerged as a powerful technology for analysis of protein conformational dynamics and ligand interactions. The regulation of transcriptional output by nuclear receptors (NRs) is driven by alterations in the conformational ensemble of the receptor upon ligand binding and previously we have shown that HDX can be used to determine a novel mechanism of ligand activation of PPARγ, detailed analysis of binding modes of ligands within the ligand binding pocket of two ER isoforms, and how ERα ligands can be classified and correlated to their pharmacology based on receptor HDX signatures. More recently, we have applied HDX to probe the conformational dynamics of intact full length nuclear receptor complexes upon interaction with DNA and coactivator proteins. These studies have demonstrated that DNA binding alters conformational dynamics of the nuclear receptor heterodimer in regions remote of the DBD. These alterations in conformational selection appear to be important for coactivator binding to the heterodimer suggesting that DNA acts as an allosteric ligand. In addition to work on NRs, the lab recently demonstrated the use of HDX for probing ligand interaction with G protein coupled receptor (GPCRs) and kinases. We have extended these studies to probe differential receptor perturbation upon interacting with functionally selective ligands, and with kinases probed both ligand and co-regulatory protein interactions. Results from these studies will be presented.

Subgroup: Intrinsically Disordered Proteins

6-Subg
A Single-Molecule Characterization of P53 Search on DNA
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The tumor suppressor p53 slides along DNA while searching for its cognate site. Central to this process is the basic C-terminal domain, whose regulatory